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Determination of Karyological Characteristics of Two Different Chickpea (*Cicer arietinum* L.) Varieties

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Abstract
In this study, the karyological characteristics of two registered chickpea varieties belonging to the Cicer arietinum species Aras and Katran were investigated. This study was carried out to determine the karyological differences in these varieties, which differ in terms of seed structure and color. Karyological observations were made by hydrolyzing and staining the fixed root tips. The chromosome number of both varieties was determined as 2n=2x=16. Both varieties were found to have the same karyotype formula (4m + 4sm). It was observed that the chromosome length of the Aras variety varied between 1.43 and 4.11 mm. When haploid chromosomes of this variety were examined, it was observed that three chromosomes had satellites on both arms of the first chromosome, the short arm of the second chromosome and the long arm of the fourth chromosome. Chromosome length varied between 1.81 and 3.89 mm in the tar variety. When the haploid chromosome set was examined, it was observed that both arms of the first chromosome and the short arms of the second, third, and sixth chromosomes had satellites. The metaphase chromosomes of the cultivars were photographed under a microscope, measured and ideograms were prepared. It was observed that there were differences between the examined varieties in terms of seed color and size, as well as some karyological differences. However, since these varieties are registered from the same species in terms of breeding, it has been observed that they are very close to each other karyologically.

Key words
Cicer, chickpea, chromosome number, karyotype

Introduction
Cicer reticulatum, defined as the wild ancestor of chickpea, has spread to large areas after being cultivated in Southeastern Anatolia and ranks second after beans among the world's legume cultivation areas. Today, it is cultivated in South Asia, West Asia, North Africa, Southern Europe, North America, South America and Australia, covering 33 countries all over the world, excluding the Antarctic continent. In South, West and East Asia, where cereal consumption is predominant, and for vegetarians who do not consume meat, chickpeas meet the need for high-quality protein (Öztürk, 2011). Chickpeas, whose homeland is the Mediterranean, contains plenty of beneficial proteins, minerals and vitamins. It is recommended that these legumes, which have high nutritional values, be consumed abundantly to increase body resistance in cold weather. Chickpeas also have properties such as cleaning the blood circulating in our body, increasing appetite, relaxing the digestive system and relieving vascular occlusion. This food, which is recommended to be consumed especially during the puerperium period because it increases breast milk, has also been reported to balance the estrogen hormone in the body (Sarıoğlan, 2022). The genus *Cicer* L. (Leguminosae) is represented in the temperate zone of the Northern Hemisphere with approximately 45 herbaceous or semi-shrub annual or perennial forms. Of these, 9 are annual and 35 are perennial taxa, of which the center of distribution is Southwest Asia and two endemic species are reported to be distributed in Morocco and Canary Islands (Van der Maesen et al., 2007). The total number of taxa, 22 of which are endemic, is 45 and the endemism rate is 48.9%. In the Flora of Turkey, the genus *Cicer* was represented by 10 species (Davis et al., 1988, Öztürk, 2011). Of these species, 4 are endemic, namely *Cicer echinospermum* P.H.Davis, *C. floribundum* Fenzl, *C. isauricum* P.H.Davis and *C. reticulatum* Ladizinsky (Davis, 1970; Davis et al., 1988). *Cicer heterophyllum* Contandr., Pamukç. & Quezel species and *C. uludereensis* Dönmez species, which are endemic to Turkey, the total number of species increased to 12 and the number of endemic species to 6, increasing the endemism rate to 50% (Öztürk, 2011). As reported by Venora et al. (1995), Dombrowsky-Sludsky (1927) was the first to report the number of chromosomes in chickpea (2n=14) and in the following years, researchers reported either 2n=14 or 2n=16 chromosomes (Rao, 1929; Dixit, 1932; Frahm-Leliveld, 1957). As a result of cytogenetic studies, this controversy has now been resolved and the chromosome number in chickpea is accepted as 2n=16. Ahmad and Hymowitz (1993) reported that the longest first chromosome of *C. arietinum*, *C. reticulatum* and *C. echinospermum* had satellites and the second chromosome of *C. reticulatum* also had satellites. Unlike the somatic karyotype analysis, detailed pachytene chromosome analysis of *C. arietinum* reported that satellites were also found on the third chromosome of this species. In this study, chromosome number and karyomorphological characteristics of two chickpea cultivars of different colors belonging to the same species (*C. arietinum*) were investigated.

Material
The seeds of the chickpea varieties used were obtained from Olgunlar agricultural company in Adıyaman province. Considering the general characteristics of chickpea varieties used in the study;

Aras (Winter Chickpeas)
Plant height 38-66 cm, upright growing, suitable for mechanized cultivation, light beige seed color, koçbaşı seed shape. Leaves are lighter colored than other varieties. The first pod height is 13-35 cm. The grains are light beige in color and it is a large grain variety. 100-grain weight is between 38.8-53.0 g.

Katran (Black Chickpea)
Plant height 33-65 cm, upright growing, suitable for machine cultivation, black seed color and dark leaves. The first pod height is 17-36 cm. It is black in color and has koçbaşı seed shape. The weight of 100 seeds is between 21.0-30.9 g.

Methods
The seeds obtained for the determination of the karyomorphological characteristics of these two cultivars were germinated on moist filter paper in petri dishes at 24°C. When the germinated seeds reached a length of 1-2 cm (Fig.1), they were pretreated in 0.05% aqueous colchicine solution for 2 hours at room temperature and pretreated in a saturated solution of 1,4 Dichlorobenzene for 4 hours at room temperature. The root tips were then removed from the pretreatment solution and fixed in acetic alcohol (1 glacial acetic acid; 3 ethanol) solution for 24 hours at +4 °C in the refrigerator. Root tips were stored in 70% alcohol in the refrigerator for later use (Acar et al., 2022; Tasar et al., 2023). For dyeing, the root tips were hydrolyzed in 1 N HCl acid for 20 minutes in an oven at 60°C. At the end of the hydrolysis process, the root tips were stained in Feulgen dye in the dark for 1 hour at room temperature (Hayta et al., 2014; Elci, 1982). When the staining time was over, the root tips were washed and left in water. In the preparations, it was observed that 0.05% aqueous colchicine solution was not effective. For this reason, the roots first treated with 1,4 Dichlorobenzene were used and gave positive results. Preparations were made to visualize the metaphase chromosomes and photographs of the appropriate metaphase chromosomes in these preparations were taken with a Nikon E200 research microscope at 100X magnification and a Nikon Digital Sight DS Fi2 microscope camera. The centromere status of chromosomes was determined according to Levan et al. (1964). Karyotype asymmetry was determined according to Huziwara (1962) (TF%), Arano (1963) (AsK%), Syi and Rec indices according to Greilhuber and Speta (1976), A index according to Watanabe et al. (1999) and A1 and A2 indices according to Romero (1986).

Figure 1. Germinated seeds used for mitotic examination; A: Aras variety, B: Katran variety

Results and Discussion

This study was carried out to determine the karyomorphological characteristics of two different colored chickpea varieties belonging to *Cicer arietinum* species. From the preparations examined, pictures of the cells suitable for karyological measurements were taken and the values of the long arm, short arm, and length of each chromosome, total length, relative length, arm ratio and centromere indices and chromosome asymmetry indices are given below.

Aras variety

In this study, the diploid chromosome number of the Aras chickpea variety was determined as 2n=16. As a result of the measurements and calculations, it was observed that the haploid chromosomes had 4 median (m) and 4 submedian (sm) structures according to the centromere status. The total length of the chromosomes varied between 1.43-4.11 mm and the total chromosome length was 18.72 mm. Arm ratios varied between 1.16 and 2.19. Relative length ranged between 8.22 and 21.96 and centromere index ranged from 31.32 to 46.22. Three pairs of chromosomes were determined in the chromosomes of the Aras variety (Table 1, Fig. 2,3).

Table 1. Karyomorphological data of Aras variety

Number	Chromosome length (µm)	Long arm	Short arm	Arm ratio	Relative length (%)	Centromeric indices (CI)	Karyotype formula
1	4.11	2.00+0.71*	0.80+0.60*	1.93	21.96	34.08	sm
2	2.63	1.41	0.77+0.45*	1.16	14.05	46.22	m
3	2.66	1.74	0.92	1.88	14.21	34.68	sm
4	2.29	0.70+0.55*	1.04	1.20	12.26	45.39	m
5	2.09	1.27	0.82	1.56	11.14	39.09	m
6	1.97	1.35	0.62	2.19	10.53	31.32	sm
7	1.54	0.93	0.61	1.52	8.22	39.63	m
8	1.43	0.94	0.49	1.92	7.64	34.20	sm

*The length of the chromosome arm where the satellite is located

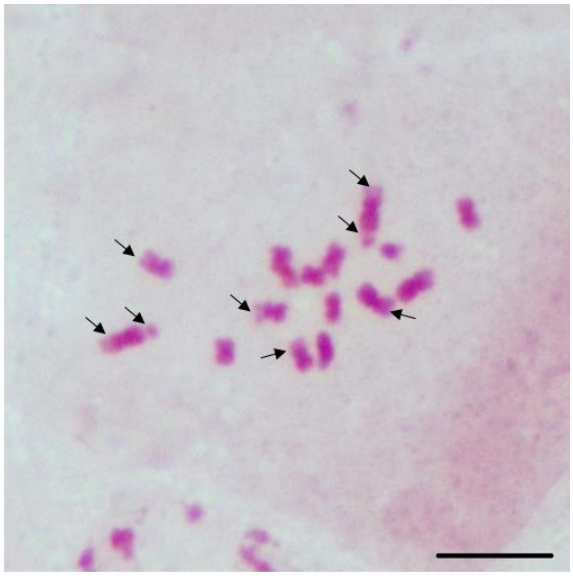

Figure 2. Metaphase chromosomes of the Aras variety (2n=16), Scala bar 10 µm.

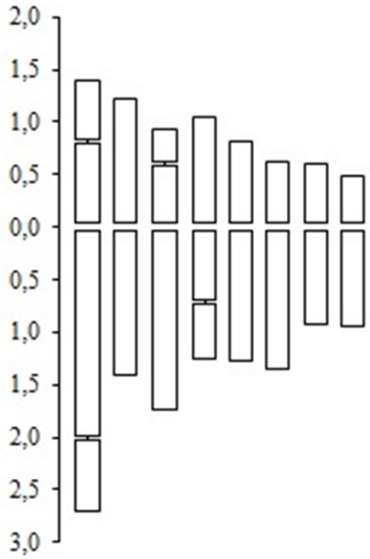

Figure 3. Ideogram of haploid metaphase chromosomes of the Aras variety

Katran variety

As a result of the karyological observations, the chromosome number of the Katran chickpea variety was determined as 2n=16. When the karyotype formula of the Katran variety is examined, it is seen that it has chromosomes with 4 media region (m) and 4 submedian (sm) centromeres. Chromosome lengths ranged between 1.81 and 3.89 mm and total chromosome length was determined as 19.45 mm. The arm ratio of the chromosomes reduced to haploid varies between 1.08 and 2.21. Relative length varied between 9.33 and 19.97 mm, while centromere index varied from 31.15 to 48.00. In addition, 4 pairs of satellites were observed in the chromosomes of the Katran variety (Table 3, Fig. 4,5).

Table 2. Karyomorphological data of Katran variety

Number	Chromosome length (µm)	Long arm	Short arm	Arm ratio	Relative length(%)	Centromeric indices (CI)	Karyotype formula
1	3.89	1.97+0.71*	0.71+0.50*	2.21	19.97	31.15	sm
2	2.89	1.50	0.78+0.61*	1.08	14.84	48.00	m
3	2.61	1.44	0.67+0.51*	1.22	13.43	44.97	m
4	2.39	1.44	0.95	1.52	12.31	39.67	m
5	2.05	1.32	0.74	1.79	10.56	35.79	sm
6	1.98	1.32	0.16+0.50*	2.02	10.18	33.08	sm
7	1.82	1.16	0.66	1.75	9.37	36.34	sm
8	1.81	1.18	0.63	1.88	9.33	34.75	sm

*The length of the chromosome arm where the satellite is located

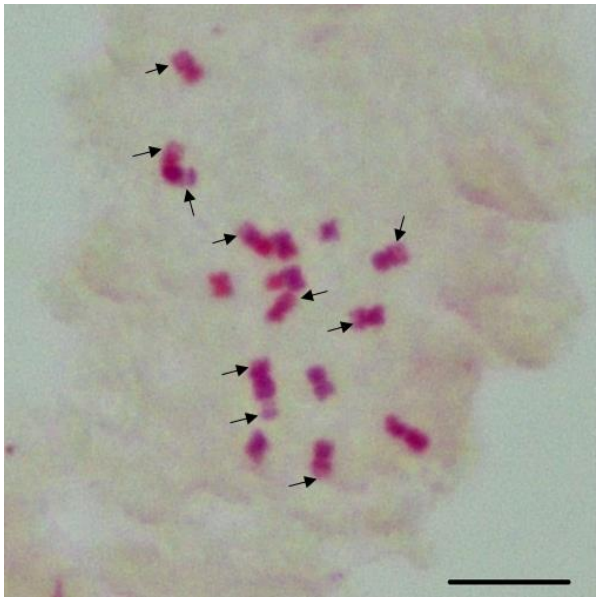

Figure 4. Metaphase chromosomes of the Katran variety (2n=16), Scala bar 10 mm.

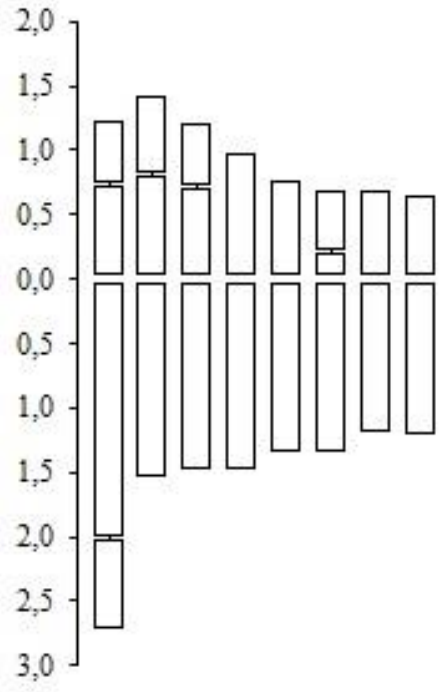

Figure 5. Ideogram of haploid metaphase chromosomes of the Katran variety

Table 3. Polyploid level, chromosome length range, total chromosome length and asymmetry index values (Rec, TF %, As K %, Syi, A, A1, A2) of different chickpea varieties (Aras and Katran).

Variety	2n	Ploidy level	Chromosome length Range	Total Chromosome Length	Rec	TF %	As K%	Syi	A	A1	A2
Aras	16	2x	1.43-4.11	18.72	56.94	37.98	62.01	61.26	0.23	0.37	0.29
Katran	16	2x	1.81-3.89	19.45	62.50	38.06	61.94	61.45	0.24	0.37	0.29

Chromosome morphologies and asymmetry indices of two different colored chickpea varieties are given in Table 3. The chromosome numbers of the varieties were determined as 2n=2x=16. Although the chromosome numbers and karyotype formulas of the varieties were the same, the Katran variety had higher values than the Aras variety in terms of both chromosome length and total chromosome length. Regarding the asymmetry indices, Rec index, TF% (total percent form) and Syi index values were higher in the Katran variety, while As K% (karyotype asymmetry index) index was higher in the Aras variety. While the A1 (intra-chromosome asymmetry) index and A2 (inter-chromosome asymmetry) index had the same value in both varieties, A (degree of karyotype asymmetry) index had a higher value in the Katran variety (Table 3). When the literature studies related to the subject studied are examined; Öner (1988) reported the chromosome number as 2n=16 in his karyological study on *Cicer arietinum* species. He also reported that chromosome lengths varied between 1.52 and 3.72 mm and a pair of satellites were determined on the largest chromosome. In this study, 4 pairs of chromosomes with satellites were observed in the Katran variety and 3 pairs of chromosomes with satellites were observed in the Aras variety. Venora et al. (1995) determined the chromosome number of *C. arietinum* species as 2n=16 and reported that the chromosome length varied between 1.03 and 3.45 mm. The karyotype formula of 3 of the 4 genotypes studied was reported as 3m + 5sm and one genotype was reported as 4m + 4sm. In this study, similar to the study of Venora et al. (1995), the karyotype formula of the cultivars in this study was determined as 4m + 4sm, which is similar to the previous studies. Öztürk (2011) reported the chromosome number as 2n=16 in his study on *C. arietinum* species.

Conclusion

In this study, the karyological characteristics of two registered chickpea cultivars belonging to *Cicer arietinum* species were determined. One of the varieties has a light beige seed color while the other one has black color. Despite the different seed structures of these varieties, the chromosome number was determined as 2n=2x=16 in both varieties. As a result of the study, since these two varieties were bred from the same species, although some differences were observed in terms of karyological characteristics (chromosome length, total chromosome length and asymmetric index), it was observed that they had close values in general. This study is similar to the results of previous studies and the satellites of the chromosomes were seen.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

O.G. karyological analysis and article writing, C.Y. material supply and article writing

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